IN THE SPECIFICATION

Please replace the indicated paragraphs in the specification with the following:

[14] FIG. 1: Design of primers and probes. The forward (P890F) and reverse (P1033R) primers anneal to highly conserved regions of the 16S rRNA gene. An internal highly conserved region was selected as the annealing site of the universal ~~Taqman~~ TAQMAN™ PCR assay probe (UniProbe), and the other internal region of highly variable sequence was selected as the annealing sites of a prototype species-specific probe for *S. aureus* (SAProbe).

[18] FIG. 5. Design of primers and probes for ~~TaqMan~~ TAQMAN™ PCR assay. The primer and probe sequences shown are as follows:

TGGAGCATGTGGTTTAATTCGA (SEQ ID NO: 5);
CCTTNTGACAACTCTAGAGATAGAGCCTTCCC (SEQ ID NO: 6);
TGCATGGYTGTCGTCAGCTCGTG (SEQ ID NO: 7);

TGTTGGGTTAAGTCCCGCA (SEQ ID NO: 8);

TGGAGCATGCGGTTTAATTCGA (SEQ ID NO: 9);
CCACNAGAACTTTCCAGAGATGGATTGGTGCC (SEQ ID NO: 10);
CCTANAGAAGTTTGCAGAGATGCAGATGTGCC (SEQ ID NO: 11);
CCAGNTGAACTTTGCAGAGATGCATTGGTGCC (SEQ ID NO: 12);
CTACNGGAATCCTCCGGAGACGGAGGAGTGCC (SEQ ID NO: 13);
CCACNGGAAGTTTTCAGAGATGAGAATGTGCC (SEQ ID NO: 14);
CCTCNTGACCCCTCTAGAGATAGAGTTTTCCC (SEQ ID NO: 15);
CCTTNGGACAACTGCAGAGATAGAGTCTTCCC (SEQ ID NO: 16);
CCCTNTGACGACTCTAGAGATAGAGTNTTNCN (SEQ ID NO: 17);
CCTTNTGACCCTTCTAGAGATAGAAGTTTCCC (SEQ ID NO: 18);

GGTGGTTGCGGATCGCAGAGATGCTTTTCCTC (SEQ ID NO: 19);
ATATNGGATATAGTTAGAGATAATTATTCCCC (SEQ ID NO: 20);
CCTTNTGACAACCCTAGAGATAGGGCTTCTC (SEQ ID NO: 21);
CCACAGAATTTGGCAGAGATGCTAAAGTGC (SEQ ID NO: 22);
CCAGCTGATCACTCTAGAGATAGAGAGTGCCT (SEQ ID NO: 23);
NGCATNGYTGTCGTCAGCTCGTG (SEQ ID NO: 24).

[33] The 16S rRNA gene sequences from a variety of bacterial species were obtained from GenBank. Sequence data were obtained using the program Entrez (see list below). The sequences were aligned using the program ClustalW from the European Bioinformatics Institute (http://www.ebi.ac.uk/clustalw.htm). Two regions of highly conserved sequences, separated by both an internal region of highly variable sequence as well as another adjacent internal region of highly conserved sequence, were selected as the universal primer annealing sites. The internal highly conserved and highly variable sequences were used as the annealing sites of conserved and species-specific ~~Taqman~~ TAQMAN™ PCR assay probes, respectively (Figure 1).

[34] The primers and ~~Taqman~~ TAQMAN™ PCR assay probes were designed according to the guidelines in the ABI Primer Express software program (PE Applied Biosystems, Foster City, CA). This program selects probes and primer sets with optimized melting temperatures, secondary structure, base composition, and amplicon lengths. The forward primer (p890F) and reverse primer (p1033R) amplify a fragment of 162 bp spanning nucleotides 890 to 1051 of the *S. aureus* 16S rRNA gene (Table 1). The universal ~~Taqman~~ TAQMAN™ PCR assay probe, or UniProbe, was labeled with the reporter dye VIC at the 5'end and the quencher dye TAMRA at the 3' end, and has the sequence which is the reverse complement of nucleotides 1002 to 1024 of the 16S rRNA gene (GenBank Accession no. AF015929) (Table 1). A *S. aureus*-specific probe, or SAProbe, was designed as the species-specific probe. The SAProbe was labeled with a different reporter dye, FAM, at the 5' end and the same quencher dye at the 3'prime end, with the sequence which spans nucleotides 945 to 978 of the *S. aureus* 16S rRNA gene (GenBank

Accession no. AF015929) (Table 1). The probes were designed to anneal to opposite strands of the template DNA. The primers and probes were manufactured by PE Applied Biosystems.

Replace the following heading to paragraph [35] with the following heading:

PCR master mix and fluorogenic-probe based PCR (~~Taqman~~ TAQMAN™ PCR assay).

[35] Reactions were performed in 50 µl volumes in 0.5-ml optical-grade PCR tubes (PE-Applied Biosystems). PCR master mix was prepared from the ~~Taqman~~ TAQMAN™ PCR assay Core Reagent Kit (PE-Applied Biosystems). The master mix was comprised of 200 µM (each) of dATP, dGTP, dUTP, dCTP, 0.5 U of AMPERASE™ ~~AmpErase~~ UNG (uracil-N-glycosylase), 2.5 mM $MgCl_2$, 1X ~~Taqman~~ TAQMAN™ PCR assay Buffer A, 900 nM of each primer, and 100 nM of each fluorescent labeled probe (UniProbe and/or SAProbe). Template DNA, 2 U of ~~AmpliTaq Gold~~ AMPLITAQ GOLD™ DNA Polymerase (PE-Applied Biosystems), and water were added to give a final volume of 50 µl for each sample. The fluorogenic-probe based PCR, or ~~Taqman~~ TAQMAN™ PCR assay, was performed using the ABI 7700 Sequence Detection system (PE-Applied Biosystems). The cycling conditions used were as follows: 50° C for 2 min, 95° C for 10 min, followed by 40 cycles at 95° C for 15 seconds and 60° C for 1 min each. All PCR reactions were performed in triplicate.

[37] An ultrafiltration step, using the Amicon ~~Microcon~~ MICROCON YM-100™ centrifugal filter device (Millipore Corporation, Bedford, Mass.) was utilized for filtering the PCR reaction mix prior to addition of template DNA. The PCR reaction mix that underwent ultrafiltration included the PCR master mix and ~~AmpliTaq Gold~~ AMPLITAQ GOLD™ DNA Polymerase. This filtration device prevents the passage of potential contaminating double stranded DNA of 125 base pairs or greater. The PCR reaction mix was spun at 100 x g for 30 minutes for decontamination purposes.

[40] The restriction endonuclease, MboII, was selected for use in the pretreatment of the PCR master mixture on the basis of the unique location of its restriction site within the amplified region of the I6S RNA by use of the Sequencher software program (Gene Codes Corp). The MboII enzyme was chosen because it has a recognition site (5'-GAAGA$(N)_8^{\nabla}$ –3' within the amplified region of 16S rRNA which is highly conserved across species, and it has no digestion site within the probe or primer sequences. The ability of the enzyme to digest a false-positive product was demonstrated by incubating 0.20 µl of MboII with 20 µl of product at a37C for 1 hr, followed by heat inactivation of the restriction enzyme at 60°C for 90 minutes and analysis by gel electrophoresis. For pretreatment of PCR reagents, 0.20 µl of MboII was incubated with PCR master mix and DEPC water at 37°C for 1 hr, followed by 60°C for 90 minutes, before the addition of Low-DNA ~~AmpliTaq Gold~~ AMPLITAQ GOLD™ DNA polymerase (PE-ABI) and template DNA.

Replace the heading to paragraph 41 with the following:

Specificity of Universal ~~Taqman~~ TAQMAN™ PCR assay.

Table 2. Specificity of the ~~Taqman~~ TAQMAN™ PCR assay using universal primers and probes.

Isolated Microorganisms	Strain (ATCC)	~~Taqman~~ TAQMAN™ PCR assay results
Staphylococcus aureus	29213	+
Staphylococcus hominis	Clinical isolate	+
Staphylococcus epidermidis	Clinical isolate	+
Streptococcus agalactiae	Clinical isolate	+
Streptococcus pneumoniae	49619	+
Klebsiella pneumoniae	990603	+

Listeria monocytogenes	Clinical isolate	+
Enterococcus faecalis	29212	+
Escherichia coli	25922	+
Proteus mirabilis	25933	+
Chlamydia pneumoniae	Clinical isolate	+
Neisseria gonorrhoeae	Clinical isolate	+
Neisseria meningitidis	Clinical isolate	+
Haemophilus influenzae (Type A)	49247	+
Candida albicans	Clinical isolate	-

Replace the heading to paragraph 42 with the following:

Theoretical detection limit of ~~Taqman~~ TAQMAN™ PCR assay.

[42] The sensitivity of the ~~Taqman~~ TAQMAN™ PCR assay was determined by amplifying serial dilutions of eubacterial DNA. The minimal detection limit of the ~~Taqman~~ TAQMAN™ PCR assay system was defined as the amount of template DNA at which the relationship between C_T and starting template DNA became nonlinear. Serial dilutions of *S. aureus* DNA (50 ng to 5 fg) were added to PCR reactions with universal primers (p890F + p1033R) and probe (UniProbe). The results are shown in Table 3. The standard curve in which C_T values were plotted against starting template DNA is linear between 50 ng to 5 pg (Figure 2). At DNA levels below 5 pg, this relationship became non-linear, and the C_T's were similar to the C_T of the no template control (NTC). This suggested the presence of contaminating eubacterial DNA in the NTC. The minimal detection limit of the assay was thus 5 pg of *S. aureus* DNA.

Replace the heading to Table 3 with the following:

Table 3. Sensitivity of the T~~Taqman~~ TAQMAN™ PCR Assay with or without pre-filtration.

[56] The contamination problem was eventually resolved by passing PCR reagents through ~~Microcon~~ MICROCON YM-100™ centrifugal filter devices (Millipore Corporation). Of note, these filters allow decontamination of all PCR reagents, including UNG, Taq polymerase, primers, and probes, which was not possible using other methods, such as DNAase treatment. Although Centricons have been employed for decontamination purposes in the past, heretofore, their adequacy in the context of real-time PCR systems had not been studied (16). With prefiltration, the PCR efficiency of the system was not reduced. In addition, with significant reduction in background contamination, the prefiltration step improved the minimum detection limit of the assay from 5000 fg to 50 fg of *S. aureus* DNA. The mean C_T of the negative-control was 40. In our experience, the C_T values of negative controls, although consistently above 35, were variable. These results were not unexpected since greater sampling errors are encountered at low starting template concentrations (12).